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EFFECT OF SELECTED CHEMICALS ON MOLD DEVELOPMENT DURING CONIFER SEED STRATIFICATION AND ON SUBSEQUENT GERMINATION

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ABSTRACT

A study to determine the need for a pre-stratification treatment of conifer seed with Captan to prevent mold development was conducted at the L. A. Moran Reforestation Center and at the Humboldt Nursery. Effectiveness of alternative treatments were also evaluated. Compared to no treatment, Captan 50-W-treated and 42-S (thiram)-treated seeds were visually cleaner and no reduction in germination occurred. However, the molds that did develop on untreated seeds had no apparent effect on germination. Bleach dip or bleach soak treatments did not reduce mold development and generally reduced germination compared to no treatment. The use of non-chemical treatments to reduce mold development, including washing the seeds with water, decreasing surface moisture content after soaking, and increasing aeration during stratification, are discussed.

INTRODUCTION

Treatment of conifer seed with the fungicide Captan prior to stratification is a standard practice at the USDA Forest Service Humboldt and Placerville nurseries and at some of the California Department of Forestry nurseries. The objective of the treatment is to inhibit mold development during naked stratification in plastic bags stored at 33°F. Following a 24-to-48 hour water soak, seeds at Humboldt are normally dipped into a solution containing 1 1/2 to 2 ounces of Orthocide 4 Flowable (37.2% Captan; Chevron Chemical Company)/ gallon of water; Placerville has used 1/2 ounce of Orthocide 4/gallon of water. At the Magalia State Nursery, seeds are soaked for 48 hours in a solution containing 2 cups of Captan 50-W (50% Captan; FMC Corporation)/15 gallons of water (approximately 1 1/4 ounces Captan 50-W/gallon of water).

Orthocide 4 is no longer manufactured, and the State of California Special Local Need for the use of the product on conifer seed has been transferred to Orthocide 75 (Chevron Chemical Company). Current labeling for Captan 50-W does not indicate its use as a seed protectant. In addition, all formulations of Captan were re-classified in 1984 as toxicity Category I (Danger) pesticides. The use of a closed system is required in California for the use of a liquid, but not powder, formulation of a Category I pesticide. Orthocide 75 is a liquid formulation.

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Because a closed system capable of measuring the small quantities of Captan used for conifer seed treatment is not available at Humboldt or Placerville, and because most conifer nurseries outside of California do not use a fungicide treatment prior to stratification, a study to determine the need for Captan and the effectiveness of alternative chemical treatments for preventing mold development was initiated. The objectives were to 1) determine the efficacy of selected chemical treatments in preventing mold development during stratification on conifer seed, and 2) to determine the effects of treatments on subsequent seed germination.

PROCEDURE

L. A. Moran Reforestation Center

Seed of seven conifer species (sugar pine, ponderosa pine, Douglas-fir, white fir, red fir and giant sequoia obtained from Placerville Nursery; and incense-cedar obtained from L. A. Moran Reforestation Center) were tested at the California Department of Forestry L. A. Moran Reforestation Center. The seed for each species was divided into lots of 55 seeds (50 seeds for germination tests and 5 seeds for mold examination). Each 55-seed lot was placed in a nylon mesh bag, soaked in water for 24 hours (white fir, red fir, Douglas-fir, giant sequoia, and incense-cedar) or 48 hours (sugar pine, ponderosa pine), and then subjected to one of the following five treatments:

1) No treatment (control)

2) Captan 50-W, 1 oz/gal of water; bags dipped in solution as done with production seed lots

3) 42-S (42% thiram; Gustafson Inc.),1 oz/gal of water; bags dipped in solution as done with production seed lots

4) 10% bleach (.525% sodium hypochlorite); bags dipped in solution as done with production seed lots

5) 10% bleach (.525% sodium hypochlorite); bags soaked in solution for 15 minutes

Treatments were selected on the basis of current use (Captan wettable powder formulation), substitute fungicide registered for seed treatment (42-S), or surface sterilant properties (bleach). Hydrogen peroxide, a surface sterilant seed treatment used in some Southern nurseries, was not tested here because of its caustic properties and expense.

Following the above treatments, the nylon mesh bags (165 total) were processed in the same manner as production seed lots at Humboldt and Placerville nurseries, including: hanging for 2 to 3 hours to drain, placement in plastic bags and tying loosely with wire twist, placement on racks in a cooler at a temperature just above 32°F, and ocassional turning of bags. Because the State and the Federal nurseries use different time periods for stratification, two time periods were tested for most species. Stratification time periods used were 4 weeks (L. A. Moran) and 45 days (Federal) for white fir and red fir; 4 weeks (L. A. Moran) and 60 days (Federal) for ponderosa pine and

Douglas-fir; 6 weeks (L. A. Moran use 6 weeks, Federal uses 45 days) for giant sequoia; 60 days (both) for incense-cedar; and 90 days (both) for sugar pine.

Efficacy of treatment was determined after stratification by germination of 50 seeds from each lot under standardized laboratory conditions at the L. A. Moran Reforestation Center (three, 50 seed replicates for each species, treatment, and stratification time). A representative sample of 5 seeds was selected from each bag and placed on 2% water agar. Fungi on the seeds or developing on the agar were identified to genus. Observations on degree of mold development in each bag were noted.

Humboldt Nursery

Seeds are normally soaked, treated, and stratified in nylon mesh bags containing about two pounds of seed. To duplicate this procedure rather than using only 55 seeds/bag, a second portion of the study was conducted at Humboldt Nursery. Thirty pounds of Jeffrey pine seed (the only species with large quantities of seed available) was divided into 15, 2-pound lots. Each 2-pound lot was placed in a nylon mesh bag, soaked in water for 48 hours, and then 3 bags treated with one of the same five treatments used at the L. A. Moran Reforestation Center. In addition to the five treatments, production Jeffrey pine seed dipped in Captan 400 D (Gustafson Inc.) was available. Three bags with 2 pounds of Dexon 400 D-treated Jeffrey pine seed in each bag was added as a sixth treatment. Following the six treatments, the nylon mesh bags received the same treatment normally used with production seed (hanging 2 to to 3 hours to drain, placement in plastic bags and tied with a twist, on racks in cooler just above 32°F, turning of bags every day for the first few days then once or twice a week). Efficacy of treatments after a 60-day stratification was determined by 1) recording germination of 100 randomly selected seeds from each bag (300 seeds/treatment) on water agar, and 2) noting and recording the degree of mold development in each bag. Fungi on seeds were identified to genus.

RESULTS

L. A. Moran Reforestation Center

Results of the germination tests of the seven conifer species tested following treatment and stratification are presented in Table 1. Although germination percentages among replicates in each treatment were variable, there was no inhibition of germination of any species following treatment with the chosen rate of Captan 50-W compared to the control. With the 42-S treatment, germination of red fir was increased and germination of white fir, incense-cedar, and sugar pine was decreased, but not significantly; and germination of ponderosa pine, Douglas-fir and giant sequoia was not affected. Effects of the bleach treatments on germination were highly variable, but with the exception of ponderosa pine and Douglas-fir, generally decreased germination. Bleach treatments of incense-cedar darkened the seeds, allowed extensive development of surface mold, and greatly reduced or prevented germination percentages.

TABLE 1. Germination of conifer seeds following pre-stratification treatment with chemicals.

Species	Days In Stratification	Treatment[x] 1	2	3	4	5
		Percent Germination[y]				
Red fir	28	40a[z]	50a	60a	25b	22b
	45	58ab	70a	73a	18c	39b
White fir	28	61a	60a	44ab	22b	35ab
	45	70a	61a	51ab	25b	45b
Ponderosa pine	28	99a	94a	98a	95a	97a
	60	97a	97a	97a	94a	93a
Douglas-fir	28	97a	99a	99a	70b	96a
	60	99a	98a	99a	52b	86a
Giant sequoia	43	50a	43a	45a	37a	42a
Incense-cedar	60	46a	53a	27b	0c	15b
Sugar pine	90	86a	73a	68a	60ab	83a

[x] Treatments:
1 = no treatment (control)
2 = Captan 50-W, 1 oz/gal of water; dipped in solution
3 = 42-S, 1 oz/gal of water; dipped in solution
4 = 10% bleach (.525% sodium hypochlorite); dipped in solution
5 = 10% bleach (.525% sodium hypochlorite); soaked in solution for 15 minutes

[y] Average percent germination of three, 50-seed replicates for each species.

[z] Germination percentages in each row followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Compared to no treatment, the Captan 50-W and 42-S treatments generally reduced the amount of surface mold developing on seed during the germination tests and on water agar plates. However, the mold that did develop on seeds with no treatment had no apparent effect on germination. Bleach treatments were consistently ineffective in reducing mold development compared to controls. In general, Captan 50-W-treated seeds were visually cleaner than non-treated; 42-S-treated seeds were almost as clean as Captan 50-W-treated; and the bleach treatments had little or no effect.

Germination results and observations suggest that mold development was influenced more by the conifer species than by the seed treatment. Seeds of ponderosa pine, Douglas-fir, sugar pine, and to a lesser extent giant sequoia, were relatively clean with very little or no mold developing even in the controls. Seeds of the two true fir species and of incense-cedar had moderate to heavy mold development, regardless of treatment.

Genera of fungi identified were mostly common saprophytes developing on seed coat surfaces. Trichoderma and Penicillium were ubiquitous regardless of treatment or conifer species, but less common on Captan 50-W and 42-S-treated seed. Rhizopus and Alternaria were also present on numerous seeds regardless of treatment. Genera occasionally present included Mucor, Epicoccum, Oidiodendron, Trimmatostroma, and Ulocladium. Rhizoctonia solani was present on one non-treated incense-cedar seed. The only species of Fusarium noted was F. roseum, from non-treated and 42-S-treated incense-cedar seed. With this one exception, no consistent relationship among species of conifer seed, treatment, and types of mold fungi developing were noted.

Humboldt Nursery

Compared to non-treated seed, the bleach treatments, but not the fungicide treatments, reduced germination percentages (Table 2).

Table 2. Germination of Jeffrey pine seed following pre-stratification treatment with various chemicals.

Treatment[x]	Percent Germination[y]
No treatment	97a[z]
Captan 50-W	93a
Captan 400 D	93a
42-S	97a
Bleach dip	73b
Bleach soak	80ab

[x] Treatments:
No treatment = control
Captan 50-W = 1 oz/gal water
42-S = 1 oz/gal water
Captan 400 D = 1 1/2 oz/gal water
Bleach dip = .525% sodium hypochlorite; bags dipped
Bleach soak = .525% sodium hypochlorite; bags soaked 15 minutes

[y] Average percent germination of three, 100-seed replicates.

[z] Means followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

Treatment did affect the amount of mold developing after 60 days of stratification. No mold was visible in bags containing Captan-treated (50-W or 400 D) seed. One bag of the 42-S-treated seed had no visible mold while the other two replicates had only slight mold development with a few clumps of seed stuck together from mycelial development. All bags of non-treated and bleach-treated seeds had extensive mold development and groups of 10 to 50 seeds stuck together by hyphae. Shaking or manipulation of the bags broke up most of the clumps. Although there was extensive mold in bags containing non-treated seed, germination percentages approached 100%, suggesting that the mold had no effect on germination and that lowered germination percentages of bleach-treated seed was due to the bleach and not to the molds.

Genera of fungi on the seeds were similar to those found on other conifer species tested at the L. A. Moran Reforestation Center, including Trichoderma, Penicillium, and occasional Epicoccum, Mucor, and Rhizopus.

SUMMARY AND CONCLUSIONS

Results of these trials indicate that Captan 50-W reduces mold development during stratification and has no significant effect on germination. Gustafson 42-S was nearly as effective as Captan 50-W. The bleach treatments did not reduce mold and, in addition, reduced germination. A Special Local Need for the use of Captan 50-W has been applied for, and the product should be registered for use on conifer seed in California by mid-January 1986. Gustafson 42-S is registered for use on seeds and, although it is a liquid formulation, does not require a closed system because available labeling indicates that it is a Category III Toxicity (Caution) pesticide.

Although either Captan 50-W or 42-S would reduce mold development compared to no treatment, their use in a dip prior to stratification may not be necessary in the overall nursery program. The molds that did develop in bags during these studies had no apparent affect on germination percentages. The clumping of seeds from mycelial growth that developed in the 2-pound bags may cause some physical problems with subsequent handling. The effect of the molds on seedling growth following sowing and germination were not studied and needs further observations.

If excessive mold development does cause problems in handling or after sowing, several procedures other than fungicide treatment would help reduce growth of fungi during stratification. Washing the seeds during the 24-to 48-hour water soak -- by changing the water several times, having a continuous flow of water, or by other means -- would probably remove many of the fungal spores from seed coat surfaces. Cleaner seeds such as the pines would need less washing than more contaminated seeds like true fir and incense-cedar. Decreasing the surface moisture content of the seeds after soaking and increasing aeration in the bags to reduce moisture during stratification would leave a less favorable environment for growth of surface contaminant fungi. Draining the bags for a longer period of time may be helpful. Using smaller amounts of seed in each bag would probably reduce free water and increase aeration. Smaller quantities of seed may also provide for more uniform and cooler temperatures in bags

Before Humboldt and Placerville nurseries omit a pre-stratification fungicide dip treatment, the procedure should be tested on a larger scale than used in this study. For example, the nurseries may want to split seed lots and try no fungicide treatment on a portion of the lot, but treat the remainder with Captan 50-W or 42-S. Observations on mold development on treated and non-treated portions should be recorded. The use of water washing, longer or more thorough drainage of bags, increased aeration in bags, and smaller quantities of seed in each bag could be tried on the untreated seeds and results recorded for future reference. In addition, treated and non-treated seed of selected or all conifer species could be sown in marked portions of a bed and seedling emergence and field performance determined and compared. Forest Pest Management is available to assist with any further studies if needed.

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